

U.S. Appln. No.: 10/561,217
Preliminary Amendment

AMENDMENTS TO THE SPECIFICATION

Attached herewith is a substitute sequence listing. Please enter the substitute sequence listing in place of the sequence listing filed with the application. No new matter has been added.

Attachment: Sequence Listing (pages 1-5).

Please replace the paragraph bridging pages 97-98 with the following amended paragraph:

For real-time quantitative PCR, forward: 5'-TGT CAC AGT CCC CAA CAC CA-3' (~~SEQ ID NO. 1~~) (~~SEQ ID NO. 3~~) and reverse: 5'-CCG AAG CAT GTG GAA AGC A-3' (~~SEQ ID NO. 2~~) (~~SEQ ID NO. 4~~) as primers, and 5'-TGT CAC CTC CCA CGG CCC G-3' (~~SEQ ID NO. 3~~) (~~SEQ ID NO. 5~~) as a probe were used. The probe was labeled at its 5'-end with fluorescence dye FAM, and its 3'-end with fluorescence dye TAMRA. Twenty-five µL of reaction mixture was prepared with 2.5 ng of cDNA prepared as described above, 1x Taqman Universal master mix (Applied Biosystems), 500 nM each of the forward and the reverse primers, and 200 nM of the probe. PCR condition was as follows: 1 cycle at 50°C for 2 minutes, 1 cycle at 95°C for 10 minutes, and 40 cycles at 95°C for 15 seconds and at 60°C for 1 minutes. Gene expression level was detected by GeneAmp 5700 Sequence Detection System (Applied Biosystems) in reaction tubes composed of MicroAmp optical 96-well reaction plate (Applied Biosystems) and MicroAmp optical cap (Applied Biosystems). Fluorescence signals were detected according to the manufacturer's instruction (Christian A. Heid, et al., in "Genome Research", 1996, Vol.6, pp.986-994). Serially 10-fold diluted plasmid DNA (3.5×10^6 , 3.5×10^5 , 3.5×10^4 , 3.5×10^3 , 3.5×10^2 and 3.5×10 molecules/well, extracted from Escherichia

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coli/SMINT2010324 host cells, which is described in Test Example 2) was used to draw a standard curve for the expression analysis.